European Journal of Medicinal Chemistry 65 (2013) 376–380

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Evaluation of *Jatropha isabelli* natural products and their synthetic analogs as potential antimalarial therapeutic agents

Victor Hadi, Megan Hotard, Taotao Ling, Yandira G. Salinas, Gustavo Palacios, Michele Connelly, Fatima Rivas*

Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678, USA

ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form
9 April 2013
Accepted 11 April 2013
Available online 23 April 2013

Keywords:
Jatropha isabelli
Jatrophone
Conjugated additions
Antimalarial agents
Plasmodium falciparum

ABSTRACT

Protozoal diseases such as malaria are a leading world health concern. We screened a library of fractionated natural products to identify new potential therapeutic leads and discovered that jatrophone (a product of *Jatropha isabelli*) exerts significant activity against *Plasmodium falciparum* strains 3D7 and K1. A focused jatrophone-scaffold library was synthesized to evaluate jatrophone's mode of action and identify more selective analogs. Compounds **25** and **32** of this natural product–inspired compound library exhibited micromolar EC_{50} values against strains 3D7 and K1, thus providing a new antimalarial molecular scaffold. Our report describes an efficient derivatization approach used to evaluate the structure–activity relationship of jatrophone analogs in search of potential new antimalarial agents.

Published by Elsevier Masson SAS.

Introduction

Malaria is among the most deadly protozoal diseases, yet only a limited number of therapeutic agents are available. Malaria is endemic to more than 100 nations and remains one of the main leading causes of death in children less than five years of age worldwide [1]. Malaria is prevalent across the globe but is localized in Africa, where its annual mortality rate is high. Although several factors are responsible for the high malaria mortality rate, the major contributors are poor vector control and the rise of drug resistance [1]. In humans, malaria is caused by four plasmodium species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *and Plasmodium malariae*), the most virulent of which is *P. falciparum* [2].

The rise of multidrug-resistant strains poses a serious threat, as the scaffold diversity of the available antimalarials is limited. Five classes of antimalarial compounds are available for therapeutic use. Two of these are chemically related — the 4-aminoquinolines (chloroquine, quinine) and 8-minoquinolines (primaquine) — while proguanil, atovaquone, and artemisinin derivatives are chemically distinct [2,3]. The World Health Organization has recommended a combination regimen based on artemisinin [1], which is the only agent *P. falciparum* is susceptible to. Resistance is expected to emerge with greater use of artemisinin derivatives [3–6], and the need for new antimalarial scaffolds is therefore more urgent. Here we report our screening of a terrestrial natural product to identify potential antimalarial lead compounds and our evaluation of jatrophone analogs as potential therapeutic agents.

Natural products have provided a direct source of therapeutic agents and a basis for drug development for the past 60 years [7]. Nature continues to provide useful structural architectures that feature selective chemical space that can potentially access new biological targets. We initially screened 150 continental American terrestrial plants with recorded ethnopharmacological properties by utilizing automated high-throughput fractionation methods [8]. This screen identified several validated hits. The natural product selected for follow-up studies was jatrophone, a compound isolated from the Paraguayan plant "yaguaroba" or *Jatropha isabelli Muell. Arg.*, belonging to the Euphorbiaceae family and popularly used medicinally [9]. When jatrophone was first structurally elucidated [10–13], it showed promising anticancer and anti-gastrointestinal properties, but its narrow therapeutic window has impeded its advancement to the clinic [12–14]. Other terpene compounds have been isolated from the rhizomes of *Jatropha isabelli*, primarily those

Abbreviations: PAMPA, parallel artificial membrane permeability assay; mCPBA, meta-chloroperoxybenzoic acid; NBS, N-bromosuccinimide; [bmim][BF_4], 1-butyl-3-methylimidazolium tetrafluoroborate.

* Corresponding author. Tel.: +1 901 595 6504; fax: +1 901 595 5715.
E-mail address: Fatima.rivas@stjude.org (F. Rivas).

0223-5234/$ – see front matter Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.ejmech.2013.04.030

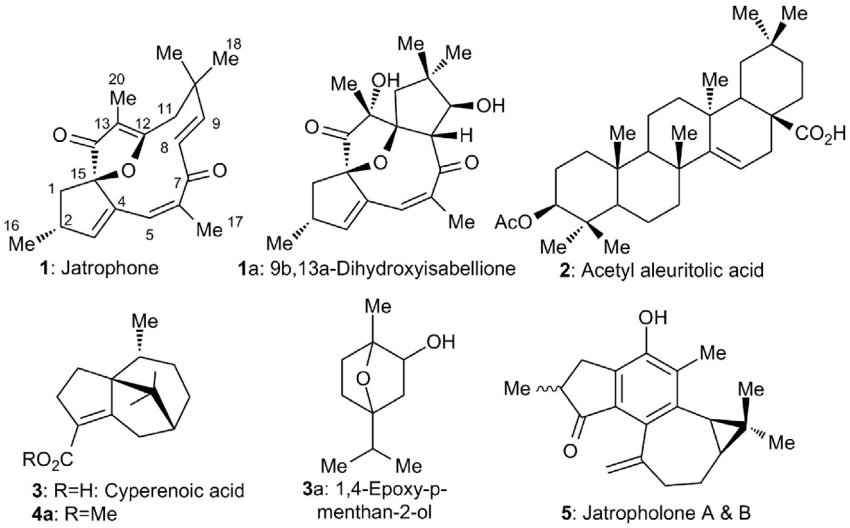

Fig. 1. Chemical constituents of *Jatropha isabelli*.

shown in Fig. 1 [14]. We isolated compounds **1**, **2**, **3**, and **5** from *Jatropha isabelli* by using a modified extraction method, but the reported terpenes (**1a** and **3a**) were not detected; jatrophone (**1**) was the main component isolated.

Jatrophone belongs to a large family of terpenes that were isolated from *Jatropha* diterpenes and that have a broad range of biological activities, including antitumor, cytotoxic, anti-inflammatory, molluscidal, and fungicidal properties [9]. Jatrophone is a macrocyclic diterpene featuring a unique oxaspiro center plus several electrophilic centers; its chemical features place it in a novel class of antimalarial scaffolds. Although jatrophone's specific biological target(s) remains elusive, its potent biological activity warrants further study. Before further derivatization, ADME (absorption, distribution, metabolism and excretion) studies were performed to evaluate jatrophone's physicochemical properties. The compound displayed good solubility (20 μg/mL at pH 3 or pH 7) and membrane permeability (5.25×10^{-4} cm/s in PAMPA assay) and a reasonable efflux ratio of 1.5 in CACO cells. Its clearance was rapid as indicated by its short half-life in human and mouse hepatic microsomes – $t_{1/2} = 0.10$ h and $t_{1/2} = 0.09$ h, respectively – and in plasma: $t_{1/2} = 0.13$ h and $t_{1/2} = 0.21$ h, respectively. Jatrophone showed the same (74%) binding affinity to human and mouse plasma proteins [15,16].

To investigate jatrophone's antimalarial structure–activity relationship (SAR), we synthesized a focused compound library and evaluated the compounds' activity against both chloroquine-sensitive (3D7) and chloroquine-resistant (K1) parasites by following an established protocol [17]. Jatrophone's highly conjugated system allows it to readily undergo 1, 2-nucleophilic additions as well as 1,4-Michael additions induced by either Grignard reagents or activated heteroatoms (S, N, and O). Previous studies of jatrophone with propanethiol suggested that its mechanism of action involves a retrograde Michael reaction that unmasks a highly electrophilic C8–C9 enone double-bond set to covalently capture the sulfhydryl group [10].

It is plausible that available protein-bound thiol groups present in the Malaria parasite react similarly with jatrophone. In cancer biology studies, selective glutathione depletion by Michael acceptors sensitized hepatocytes to hypoxia, making glutathione depletion a promising potential antitumor strategy [18]. To examine whether jatrophone was reacting or depleting glutathione, we studied the reaction of jatrophone with reduced glutathione and with thiophenol. The Michael adduct **7** was obtained from the reaction with glutathione, which was the limiting reagent. When more than half an equivalent of glutathione was added, complex compound mixtures were observed. In contrast, reaction with

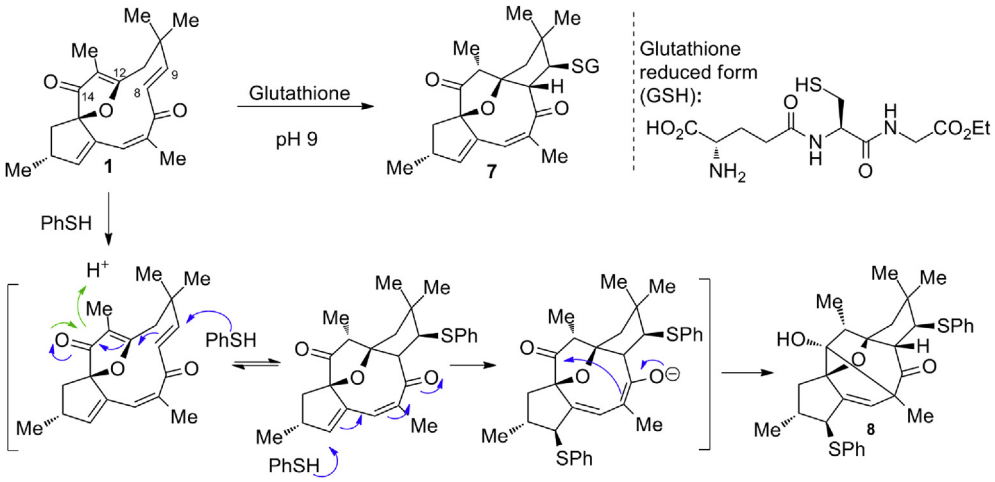

Fig. 2. Conjugated cascade cyclization reactions of jatrophone with sulfhydryl groups.

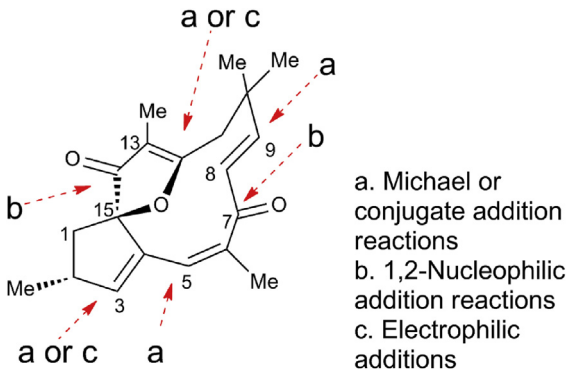

Fig. 3. General synthetic approaches to jatrophone analogs.

thiophenol yielded only the stable trans-annular conjugated Michael product **8** (Fig. 2), illustrating the ease with which the small, electron-rich thiol group was added at multiple sites. These findings suggest that both size and electronic properties influence the outcome of addition of thiol-bound reagent, but additional biological evidence is needed to support whether jatrophone is indeed reacting with a thiol-group as its mechanism of action.

With this preliminary information, we proceeded to investigate jatrophone's potential as an antimalarial agent and seek to broaden its therapeutic window. Taking the systematic approach illustrated in Fig. 3, we used conjugated additions, including 1,2 and 1,4-Michael additions at C7, C9, and C12, C14, to impede the binding of endogenous thiol groups and probe if they kept their activity. Overall reduction reactions of the jatrophone olefin systems was expected to limit its reactivity, and electrophilic additions were made to help probe jatrophone's chemical space and to investigate if they retain biological activity. Each individual constituent of *Jatropha isabelli* was also evaluated for antimalarial properties to anticipate any synergism attributable to traces of other compounds during the fractionation process.

As shown in Fig. 4, the exploration commenced with the hydrogenation of jatrophone, affording compounds **9** and **10**. Several attempts to selectively conduct epoxidation [19] across C8–C9 were unsuccessful due to competing side reactions. However, epoxidation of jatrophone at C3–C4 by using mCPBA resulted in the thermally stable compound **11**, presumably through oxidative rearrangement at C12–C13. A Wittig reaction of the enone at C-7 afforded the expected exocyclic olefin **12**, and deoxygenation of the allylic hydroxyl at C-7 with a hydride source (Et_3SiH) in the presence of a Lewis acid ($BF_3.Et_2O$) afforded product **12a** [20].

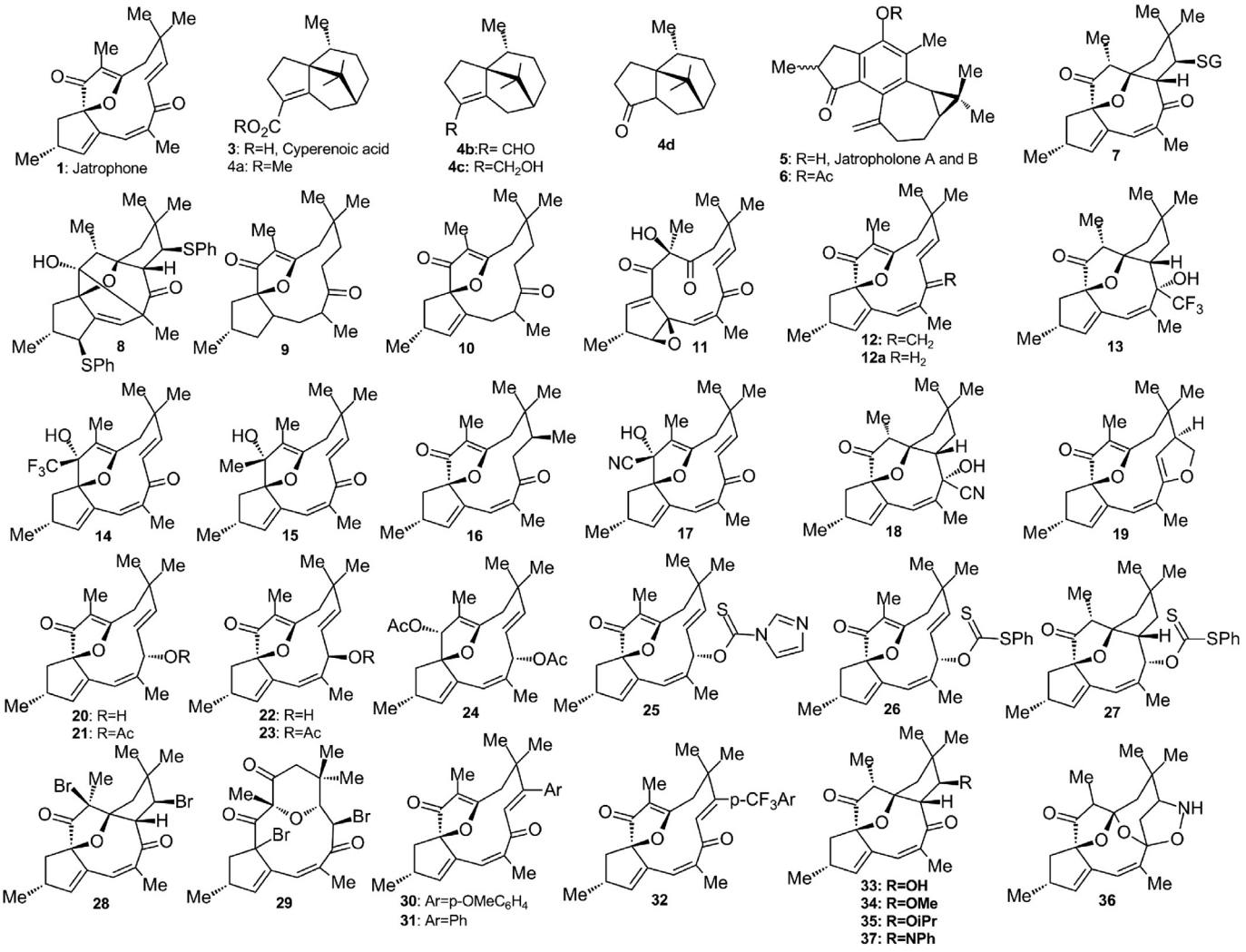

Fig. 4. Focused jatrophone compound library **1**–**37**.

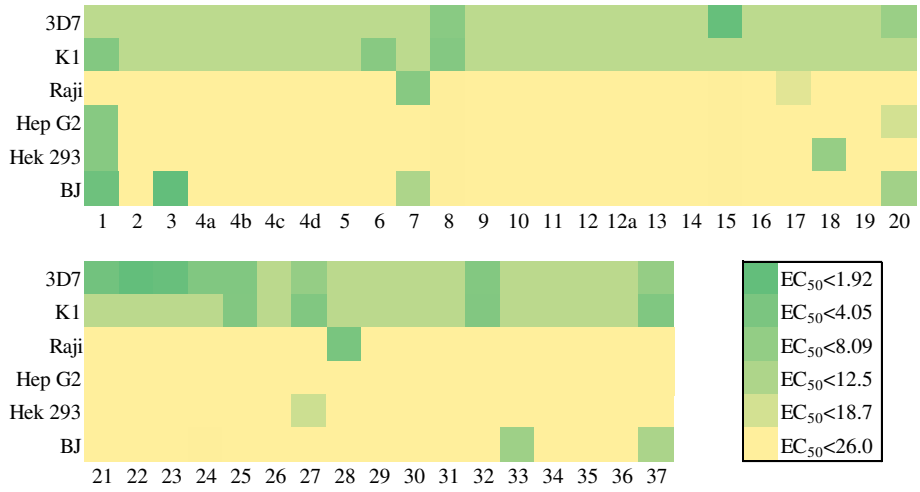

Fig. 5. Heat map of antimalarial activity and cytotoxicity of compounds **1**–**37**.

The reaction of jatrophone with CF_3SiMe_3 in the presence of a catalytic quantity of CsF proceeded predominantly via 1,2-addition, yielding a 2:3 mixture of compounds **13** and **14**, respectively, after hydrolysis [21]. A methyl Grignard reaction yielded the 1,2-addition product **15** and the 1,4-conjugated addition compound **16** as byproducts. In view of the high affinity of silicon for fluoride anion, jatrophone was treated with trimethylsilyl cyanide, and CsF, providing compounds **17** and **18** [22].

Metal-free cyclopropanation reactions of jatrophone with diazomethane afforded only dihydrofuran product **19**, via carbene addition at C9 with simultaneous enolate treatment to release nitrogen gas [23]. Selective 1,2-reduction of enone C7 with sodium borohydride in combination with $CeCl_3$ resulted in the expected diastereoisomers **20** and **22** (3:1 ratio in favor of equatorial hydride attack) and trace amounts of jatrophone reduced at both C7 and C14. Acetylation of the resulting hydroxyl groups yielded compounds **21**, **23**, and **24**.

Further derivatization of compound **20** to the corresponding imidazole-1-carbothioate by treatment with 1,1′-thiocarbonyldiimidazole provided compound **25**, and treatment with phenyl chlorodithioformate in the presence of 4-dimethyaminopyridine afforded compounds **26** and **27**.

Electrophilic reactions, particularly bromination, were of high interest given the fact that bromination often enhances the bioactive properties of organic compounds [24]. Because the use of molecular bromine had been reported to afford mixtures [11], we used other bromine-containing reagents, such as N-bromosuccinimide (NBS). As treatment of jatrophone with NBS in carbon tetrachloride produced small quantities of compound **28**, we sought an alternative reaction condition. Jatrophone was then treated with NBS in ionic liquid medium [bmim][BF_4] (known to increase polarization of the N–Br bond, enhancing the reagent's reactivity and shortening the reaction time), yielding compound **29** and the starting material [24].

Having evaluated several functionalities of jatrophone, we turned our attention to electrophilic reactions that induce subtle changes in the overall electronic properties of the parent compound, but changes in the overall chemical space. Stereoselective synthesis of cyclic, substituted olefins by Heck reaction of organic halides with an α,β-unsaturated system are fairly uncommon. However, previous studies had shown that through a complex mechanism, 1,2-disubstituted, electron-poor olefins can undergo a Heck-type reaction in the presence of palladium (II), Ag_2CO_3 as the base, and a large excess of the iodo-aromatic donor [25]. In fact, Heck cross-coupling reactions of jatrophone with p-iodoanisole, iodobenzene, and iodo-benzotrifluoride readily afforded compounds **30**, **31**, and **32**, respectively. Finally, addition of non-carbon soft nucleophiles (H_2O, MeOH, iPrOH) to the C9 of jatrophone proceeded efficiently to afford compounds **33**, **34**, and **35**. Selective hydroxylamine or aniline addition at C9 produced compounds **36** and **37** respectively, albeit in poor yields.

Some of the presented reactions did not proceed in good yields due to the high complexity of the starting material, and its instability under basic and high temperature conditions.

The heat map corresponding to our antimalarial strains and cytotoxicity mammalian cell lines (Raji, HepG2, HEK293, and BJ) results for compounds **1**–**37** are illustrated in Fig. 5. These results indicated that the olefin C8–C9 of jatrophone played an important role in its activity, and subtle changes at this bond allowed the development of selective compounds. Although most jatrophone analogs exerted antimalarial effects against both the 3D7 and K1 strains, few compounds (compounds **8**, **15**–**20**, **22**–**23**, **29** and **37**) were more potent than the parent compound. Although more potent compounds were needed, we were interested primarily in compounds more effective against the K1 (chloroquine-resistant) malaria strain. Compounds **8**, **25**, **27**, **32**, and **37** met this criterion although they had lost their extended conjugation system, indicating that the overall scaffold contributes to compound activity.

Surprisingly, addition of electron-rich aromatic groups at C-9 (compounds **30**–**31**) had only a moderate effect on antimalarial activity and showed no cytotoxicity to the tested mammalian cell lines. However, compound **32** was a substantial lead, with potency similar to that of jatrophone against both malaria strains (EC_{50}: 6.07 and 5.8 μM for 3D7 and K1, respectively) and low cytotoxicity. Further, its physicochemical properties did not differ substantially from those of the parent compound (solubility, 17 μg/mL; permeability, 1.06×10^{-4} cm/s in PAMPA assay). Together, these jatrophone analogs provided an activity profile of the overall chemical space. While the substitution pattern was important, the olefin across C8–C9 proved to be instrumental to overall reactivity.

In conclusion, our systematic synthetic derivatization of the natural product jatrophone yielded the promising compound leads **25** and **32**, which displayed good antimalarial activity and lower cytotoxicity than that of jatrophone in mammalian cells. Our findings warrant further studies to identify the biological target of this highly functionalized molecular scaffold, which possesses a chemical space different from those of known antimalarial therapeutic agents.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

This study was supported by the American Lebanese Syrian Associated Charities (ALSAC). The authors thank Ing. Mariza C. Quintana Centurion of the Museo Nacional de Historia Natural del Paraguay for assistance with the ethnobotanical survey and collection and identification of *Jatropha isabelli*. We also thank the High-Throughput Analytical Chemistry Core Facility at St. Jude Children's Research Hospital for analytical support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.04.030.

References

[1] World Health Organization, World Malaria Report (2011). Geneva, http://www.who.int/malaria/world_malaria_report_2011/en/ (accessed 13.01.2013).
[2] E.I. Lueze, S.L. Croft, D.C. Warhurst, Activity of pyronaridine and mepacrine against twelve strains of *Plasmodium falciparum in* vitro, J. Antimicrob. Chemother. 37 (1996) 511–518.
[3] C.J. Sutherland, S.D. Polley (Eds.), Genetics and Evolution of Infectious Diseases, Elsevier, 2011, pp. 607–627.
[4] M.E. Sinka, J.M. Bangs, S. Manguin, M. Coetzee, C.M. Mbogo, J. Hemingway, A.P. Patil, W.H. Temperley, P.W. Gething, C.W. Kabaria, R.M. Okara, T.V. Boeckel, H.C.J. Godfray, R.E. Harbach, S.I. Hay, The dominant vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis, Parasit. Vectors 3 (2010) 117.
[5] S. Bröer, K. Kirk, Chloroquine transport via the malaria parasite's chloroquine resistance transporter, Science 325 (2009) 1680–1682.
[6] V. Lakshmanan, P.G. Bray, D. Verdier-Pinard, D.J. Johnson, P. Horrocks, R.A. Muhle, G.E. Alakpa, R.H. Hughes, S.A. Ward, D.J. Krogstad, A.B. Sidhu, D.A. Fidock, A critical role for PfCRT K76T in *Plasmodium falciparum* verapamil-reversible chloroquine resistance, EMBO J. 24 (2005) 2294–2305.
[7] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs over the 30 years from 1981 to 2010, J. Nat. Prod. 75 (2012) 311–335.
[8] Y. Tu, C. Jeffries, H. Ruan, C. Nelson, D. Smithson, A.A. Shelat, K.M. Brown, X.C. Li, J.P. Hester, T. Smillie, I.A. Khan, L. Walker, L.,K. Guy, B. Yan, An automated high-throughput system to fractionate plant natural products for drug discovery, J. Nat. Prod. 73 (4) (2010) 751–754.
[9] R.K. Devappa, H.P.S. Makkar, K. Becker, *Jatropha* diterpenes: a review, J. Am. Oil Chem. Soc. 88 (2011) 301–322.
[10] J.P. Mallari, W.A. Guiguemde, R.K. Guy, Antimalarial activity of thiosemicarbazones and purine derived nitriles, Bioorg. Med. Chem. Lett. 19 (2009) 3546–3549.
[11] S.M. Kupchan, C.W. Sigel, J.M. Matz, C.J. Gilmore, R.F. Bryan, Structure and stereochemistry of jatrophone, a novel macrocyclic diterpenoid tumor inhibitor, J. Am. Chem. Soc. 98 (1975) 2295–2300.
[12] M.D. Taylor, A.B. Smith, G.T. Furst, S.P. Gunasekara, C.A. Bevelle, G.A. Cordell, N.R. Farnsworth, S.M. Kupchan, H. Uchida, A.R. Branfman, R.G. Dailey, A.T. Sneden, New antileukemic jatrophone derivatives from *Jatropha gossypifolia*: structural and stereochemical assignment through nuclear magnetic resonance spectroscopy, J. Am. Chem. Soc. 105 (1983) 3177–3183.
[13] G. Schmeda-Hirschmann, I. Razmilic, M. Sauvain, C. Moretti, V. Munoz, E. Ruiz, E. Balanza, A. Fournet, Antiprotozoal activity of Jatrogrossidione from *Jatropha grossidentata* and Jatrophone from *Jatropha isabellii*, Phytother. Res. 10 (1996) 375–378.
[14] M. Pertino, G. Schmeda-Hirschmann, J.A. Rodriguez, C. Theoduloz, Gastroprotective effect and cytotoxicity of terpenes from the Paraguayan crude drug "yagua rova" (*Jatropha isabelli*), J. Ethnopharmacol. 111 (2007) 553–559.
[15] L. Di, E.H. Kerns, X.J. Ma, Y. Huang, G.T. Carter, Applications of high throughput microsomal stability assay in drug discovery, Comb. Chem. High Throughput Screen. 11 (2008) 469–476.
[16] A.M. Marino, M. Yarde, H. Patel, S. Chong, P.V. Balimane, Validation of the 96 well Caco-2 cell culture model for high throughput permeability assessment of discovery compounds, Int. J. Pharm. 297 (2005) 235–241.
[17] K. Mitachi, Y.G. Salinas, M. Connelly, N. Jensen, T. Ling, F. Rivas, Synthesis and structure–activity relationship of disubstituted benzamides as a novel class of antimalarial agents, Bioorg. Med. Chem. Lett. 22 (2012) 4536–4539.
[18] J.M. Lluis, A. Morales, C. Blasco, A. Colell, M. Mari, C. Garcia-Ruiz, J.C. Fernandez-Checa, Critical role of mitochondrial glutathione in the survival of hepatocytes during hypoxia, J. Biol. Chem. 280 (2005) 3224–3232.
[19] M.A. Gonzalez, S. Ghosh, F. Rivas, D. Fischer, E.A. Theodorakis, Synthesis of (+) - and (-)-isocarvone, Tetrahedron 45 (2004) 5039–5041.
[20] D.G. Batt, G.D. Maynard, J.J. Petraitis, J.E. Shaw, W. Galbraith, R.R. Harris, 2-Substituted-1-naphthols as potent 5-lipoxygenase inhibitors with topical anti-inflammatory activity, J. Med. Chem. 33 (1990) 360–370.
[21] R.P. Singh, J.M. Shreeve, Nucleophilic trifluoromethylation reactions of organic compounds with (trifluoromethyl)trimethylsilane, Tetrahedron 56 (2000) 7613–7632.
[22] J. Yang, Y. Wang, S. Wu, F.X. Chen, The highly efficient 1,4-addition of TMSCN to aromatic enones catalyzed by CsF with water as additive, Synlett (2009) 3365–3367.
[23] H. Lebel, J.F. Marcoux, C. Molinaro, A.B. Charette, Stereoselective cyclopropanation reactions, Chem. Rev. 103 (2003) 977–1050.
[24] J.S. Yadav, B.V.S. Reddy, G. Baishya, S.J. Harshavardhan, C.J. Chary, M.K. Gupta, Green approach for the conversion of olefins into *vic*-halohydrins using *N*-halosuccinimides in ionic liquids, Tetrahedron 46 (2005) 3569–3572.
[25] P. Mauleon, Palladium-catalyzed cascade reaction of α,β-unsaturated sulfones with aryl iodides, Eur. J. Chem. 9 (2003) 1511–1520.